

# Clonal Variation in Morphology of *Populus* Root Systems Following Irrigation with Landfill Leachate or Water during 2 Years of Establishment

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**Abstract** Increased municipal solid waste generation in North America has prompted the use of *Populus* for phytoremediation of waste waters including landfill leachate. *Populus* species and hybrids are ideal for such applications because of their high water usage rates, fast growth, and extensive root systems. Adventitious rooting (i.e., lateral rooting from primordia and basal rooting from callus) of *Populus* is important for phytotechnologies to ensure successful plantation establishment with genotypes that thrive when irrigated with highly variable or specific contaminants. We evaluated differences in root system morphology following establishment with high-salinity municipal solid waste landfill leachate or uncontaminated well water (control). *Populus* clones (NC13460, NC14018,

NC14104, NC14106, DM115, DN5, NM2, and NM6) were irrigated during 2005 and 2006 in northern Wisconsin, USA and tested for differences in morphology of lateral and basal root types, as well as fine (0–2 mm diameter), small (2–5 mm), and coarse (>5 mm) roots. Across treatments and clones, trees averaged five roots per root type. Leachate-irrigated trees had 87% (lateral) and 105% (basal) as many roots as those irrigated with water. Leachate-irrigated trees had 96% as many fine roots as watering with irrigation water, whereas trees with leachate had 112% (small) and 88% (coarse) as many roots versus water. Despite root necrosis and regrowth in 23% of the trees, leachate irrigation did not negatively affect root diameter or dry mass. Given that adequate rooting is necessary for plantation establishment, leachate and similar waste waters are viable irrigation and fertilization sources of *Populus* crops used as feedstocks for biofuels, bioenergy, and bioproducts.

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## Introduction

Increased municipal solid waste generation in North America and throughout the world has prompted the use of *Populus* for phytoremediation of waste waters including landfill leachate [4, 15]. *Populus* species and hybrids are ideal for such applications because of their high water usage rates, fast growth, and extensive root systems [13, 16]. In areas similar to the northern Midwest of the USA, it is most economical to establish *Populus* with unrooted, dormant hardwood cuttings. Plantation success in such systems depends upon the development of adventitious roots

[30, 31]. However, in variable and potentially toxic phytotechnologic applications, the development of sufficient adventitious roots to sustain health of the trees may be reduced over traditional irrigation regimes. Therefore, it is important to assess adventitious rooting under these conditions to select favorable genotypes for testing and deployment [26, 27].

There are two categories of adventitiously derived roots from *Populus* cuttings. The first type, *lateral roots*, develops from preformed or induced root primordia receiving stimulation in response to internal and external factors that trigger cell division, differentiation, and root growth [17, 23]. The second type, *basal roots*, develops from callus at the base of the cutting following mechanical wounding during processing of the parent shoot into propagules. After field planting and exposure to necessary hormonal and environmental stimuli, callus cell proliferation differentiates into root primordia followed by basal adventitious root formation [8]. Both adventitious root types are the source of the extensive network of horizontal, vertical sinker, and fine roots of asexually propagated *Populus* clones [19].

Although previous studies have documented variability in *Populus* root system morphology under traditional irrigation and/or fertilization regimes [1, 7, 21], information about differences in rooting with wastewater systems is lacking. Nevertheless, there is an opportunity to select *Populus* genotypes with some combination of both adventitious root types to enhance the success of phytoremediation and associated technologies. Anticipated outcomes from this selection goal are to increase the root foraging area for contaminant removal, improve erosion control, and lessen impacts to groundwater. In addition, due to the effect of increased adventitious rooting on improved growth and survival, plantation yields may be improved and management expenses reduced [30].

This research advances the use of *Populus* for phytotechnologies beyond our previously published studies that included genotype selection [26], biomass accumulation [27], and tissue uptake of sodium/chloride [28] and macro- and micronutrients [29]. In the current study, we examined differences in root system morphology during 2 years of plantation establishment with irrigation and fertilization from high-salinity municipal solid waste landfill leachate or uncontaminated well water (control). The primary objective was to test for differences among eight *Populus* clones in morphology of lateral and basal adventitious root types and three diameter size classes: fine (0–2 mm), small (2–5 mm), and coarse (>5 mm). Our hypotheses were that clones would respond differently to the irrigation treatments and that clones would differ for the formation, number, and size of lateral and basal adventitious roots. These data are important because short rotation woody energy crops are

vital feedstocks for the biofuels, bioenergy, and bioproducts industries, as well as being renewable resources that provide numerous environmental services. In this study, we present practical new information for researchers and resource managers who are reliant on adventitious rooting for short rotation woody crop production systems, especially given current fossil fuel concerns and continued ecological degradation.

## Materials and Methods

Details about site description, clone selection, tree establishment, experimental design, treatment application, and elemental analyses of treatments and soils were described previously [27–29]. A summary of relevant materials and methods is discussed below, followed by specific sampling, measurements, and data analysis information that is unique to the current paper. The study was conducted at the Oneida County Landfill (municipal solid waste) located 6 km west of Rhinelander, WI, USA (45.6° N, 89.4° W). Temperature, precipitation, and growing degree days across the experimental period were reported previously [27]. The landfill soils are classified as mixed, frigid, coarse loamy Alfic Haplorthods (Padus Loam, PaB), with 0–6% slopes, and are considered well to moderately well drained with loamy deposits underlain by stratified sand and gravel glacial outwash. Preplanting and harvest soil pH and electrical conductivity, as well as concentrations of total carbon (C), nitrogen (N), phosphorus (P), potassium (K), sodium (Na), chloride (Cl), calcium (Ca), magnesium (Mg), sulfur (S), zinc (Zn), boron (B), manganese (Mn), iron (Fe), copper (Cu), aluminum (Al), and lead (Pb) were described previously [27–29].

Eight *Populus* clones belonging to four genomic groups (Table 1) were selected from 25 original genotypes during three phyto-recurrent selection cycles based on 23 traits relating to height growth, leaf development, and root initiation [26]. In this paper, we use *Populus* section names as specified by Eckenwalder [5], as well as the current classification of *P. maximowiczii* A. Henry as a subspecies of *P. suaveolens* Fischer [2, 5].

Shoots were collected during dormancy from stool beds established at Hugo Sauer Nursery in Rhinelander. Hardwood cuttings, 20 cm long, were prepared in January 2005, with cuts made to position at least one primary bud not more than 2.5 cm from the top of each cutting. Cuttings were stored at 5°C and soaked in water to a height of 15 cm for 3 days before planting on 14 June 2005. Prior to planting, the soil was tilled to a depth of 30 cm. Cuttings were planted in a split plot design with eight blocks, two treatments (whole plots), and eight clones (sub plots) at a spacing of 1.2×2.4 m (3,472 trees per hectare). Two border

**Table 1** Genomic groups and clones of *Populus* irrigated with well water (control) and municipal solid waste landfill leachate for two growing seasons in northern Wisconsin, USA

Genomic group	Clone
<i>(P. trichocarpa</i> × <i>P. deltoides</i> ) × <i>P. deltoides</i> “TD×D”	NC13460, NC14018
<i>P. deltoides</i> × <i>P. suaveolens</i> subsp. <i>maximowiczii</i> “DM”	NC14104, NC14106, DM115
<i>P. deltoides</i> × <i>P. nigra</i> “DN”	DN5
<i>P. nigra</i> × <i>P. suaveolens</i> subsp. <i>maximowiczii</i> “NM”	NM2, NM6

Authorities for the aforementioned species are *P. deltoides* Bartr. ex Marsh, *P. trichocarpa* Torr. & Gray, *P. nigra* L., *P. suaveolens* Fischer subsp. *maximowiczii* A. Henry

rows of clone NM2 were established on the perimeter of the planting and between treatment whole plots to reduce potential border effects [9]. Mechanical and hand weeding were performed weekly. Electric fencing and polyvinylchloride tubing (installed on each tree after leaf senescence in November 2005) was used to prevent mammalian-induced injury.

Water (control) from a non-impacted well located 100 m from the study area was applied three times at a rate of 3.8 L per tree to all cuttings via hand irrigation for 14 days following planting. Once established, trees were hand irrigated with either fertilized water or municipal solid waste landfill leachate that was collected weekly. Nitrogen, P, and K were added to the control water during each irrigation application at a rate equal to that of the leachate to eliminate fertilization effects. The weekly application rate in 2005 was 3.8 L per tree (eight applications; 1.9 kL total per treatment) while that applied via drip irrigation in 2006 was 22.7 L per tree (12 applications; 17.4 kL total per treatment). Treatment application was adjusted based on precipitation to prevent substantial leaching from the experimental plot. Irrigation was postponed if more than 0.5 cm of rainfall occurred within 2 days prior to watering or was expected to occur with a 40% chance or greater for 2 days following watering.

### Sampling and Measurements

#### *Well Water (Control) and Municipal Solid Waste Landfill Leachate*

Water and leachate from the same source as the irrigation treatments were sampled from the Oneida County Landfill during April and October of 2005 and 2006. The water and leachate chemistry was analyzed (Northern Lake Service, Inc., Crandon, WI, USA) using approved US Environmental Protection Agency methods. Electrical conductivity, pH, biological oxygen demand, and chemical oxygen demand, as well as concentrations of total C, N, P, K, Na, Cl, Ca, Mg, S, Zn, B, Mn, Fe, Cu, Al, and Pb were described previously [27–29]. While relatively high Cl ( $1250 \pm$

$50 \text{ mg L}^{-1}$ ) and Na ( $1200 \pm 0 \text{ mg L}^{-1}$ ) concentrations were the primary toxicity concern, heavy metals and volatile organic compounds were not detectable in the water or leachate analyses and, therefore, not a concern with respect to plant establishment and development.

#### *Root Tissues*

All trees were destructively harvested on 18 August 2006. Processing of aboveground tissues was described previously [27–29]. The root system of each tree was excavated using a mechanized tree spade that removed a uniform, conical volume of soil (diameter × depth =  $0.28 \text{ m}^3$ ). *Fine roots* (<2 mm diameter) were scored on a scale of 0–2, with 0 meaning a lack of fine roots and 2 meaning substantial fine root development. The presence of five unique traits was recorded: (1) deep, vertically oriented sinker roots, (2) mats of fine roots, (3) induced callus cell proliferation extruding from the cutting lenticels, (4) fine root clusters consisting of multiple roots initiated from the same origin on the cutting, and (5) necrotic fine root tissue dieback and subsequent regrowth.

Root systems were washed and divided into stump, lateral root, and basal root components. Lateral roots develop from latent root primordia distributed throughout the length of the original cutting, while basal roots develop from callus as a result of wounding at the base of the cutting [17, 31]. Lateral and basal roots were further categorized into *small roots* (2–5 mm diameter at the point of attachment to the stump) and *coarse roots* (>5 mm diameter) [7, 21]. Number of roots and root diameter were recorded for each root type and size. Root tissues were bulked according to root type and dried at 70°C. Stump, lateral root, basal root, and total belowground (stump + lateral + basal) dry mass was determined when values reached a constant mass.

#### *Data Analysis*

Given 78% survival at the time of harvest, six complete blocks of each treatment were included in the analyses. In

addition to fine root scoring data, number of roots and diameter data were analyzed separately by each combination of root type and size using analyses of variance assuming the split plot design described above (proc mixed [22]). Blocks (i.e., replications) were considered random in the analysis, while irrigation treatments were fixed whole plots and clones were fixed subplots. Similarly, the number and diameter data were analyzed separately by root size assuming a split split plot design with a random block effect and fixed main effects for irrigation treatment (whole plot), clone (subplot), and root type (subsubplot). Where appropriate, non-significant ( $P > 0.25$ ) interaction terms that included the block main effect were pooled into a common error term to increase precision of  $F$  tests for both models [31]. Given the fixed main effects in each model, means were evaluated rather than variances. Fisher's protected least significant difference was used to compare means, which were considered different at  $P < 0.05$ .

Allometric relationships among the number of roots, fine root scoring, and dry mass were analyzed using simple (Pearson) correlation coefficients (proc corr; [22]). Likewise, the proportion of number of roots for each combination of root type and size was used to assess allometric relationships among rooting traits for irrigation treatment  $\times$  clone interactions.

## Results and Discussion

### Mortality

The overall mortality rate across treatments was 22%, ranging from 6% (NM6) to 56% (NC13460). Fourteen trees from both treatments were dead at the time of harvest. This mortality and additional visual damage to vegetative growth due to high chloride and sodium in the leachate and soil were described previously [28].

### Fine Roots (0–2 mm)

The irrigation treatment  $\times$  clone interaction was significant for the presence and abundance of fine roots ( $P = 0.0156$ ). Leachate and control irrigation treatments did not differ for mean rating of fine roots within individual clones; however, fine root rating ranged from 0 to 1.7 (0 = no fine roots, 2 = well-developed fine roots) across treatment  $\times$  clone interactions (Table 2). Fine root allocation is typically a small proportion of total *Populus* root tissue that may be unsteady in young plantations and decrease with maturity [14]. Yet, regardless of stand age, this root category is a dynamic storage pool of forest carbon, as fine roots have been reported to be subject to rapid carbon cycling, account for a great deal of total net primary productivity, and vary greatly

**Table 2** Mean rating of fine roots (0–2 mm) of eight *Populus* clones 14 months after planting following weekly irrigation with well water (control) or landfill leachate

Clone	Irrigation treatment	
	Control	Leachate
NC13460	0.0 f	0.7 cdef
NC14018	1.3 abc	1.7 a
NC14104	1.2 abcd	1.5 ab
NC14106	0.3 ef	0.8 bcde
DM115	1.3 abc	1.2 abcde
DN5	1.0 abcde	0.3 ef
NM2	1.2 abcd	0.5 def
NM6	1.3 abc	0.7 cdef

Fine root rating scale was from 0 to 2, with zero being no fine roots and two being well-developed fine roots. Each score represents the mean of six trees. Combinations labeled with different letters were different at  $P < 0.05$

in life span [14]. Friend et al. [7] reported that fine roots comprised 35% of total root mass of a pair of 2-year-old *Populus trichocarpa*  $\times$  *Populus deltoides* "TD" hybrids, while Heilman et al. [11] reported 21–34% of total root mass was in fine roots of 4-year-old TD hybrids and their parental species. In addition, Puri et al. [21] found that 70–80% of fine root biomass of *P. deltoides* was located in the top 15 cm of soil and had increased with greater spacing between trees. Similarly, Heilman et al. [11] reported that the majority of fine roots developed in the top 18 cm of soil.

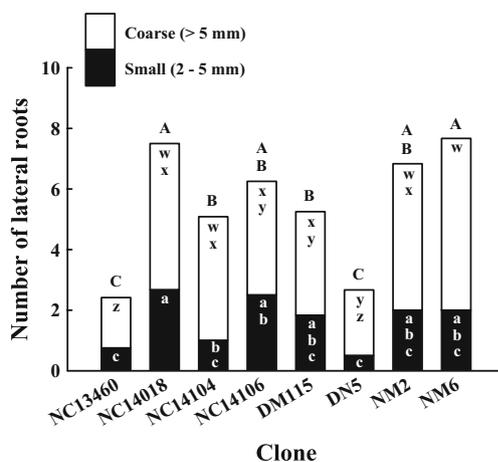
Genetic control of rooting was prevalent in the current study, with fine root production unaffected by either irrigation treatment. Trees irrigated with leachate had 96% as much fine root biomass as those receiving water. Coleman [1] reported that fertilized genotypes across species (including *P. deltoides*) averaged 21% more fine root biomass than the control and 12% greater mass with irrigation versus the control. Twenty-three percent of leachate-irrigated trees in the current study suffered from necrotic fine root tissue dieback and subsequent regrowth at the same nodal location. This rooting response appeared to result from increased salt strength and/or chemical constituents imposed by the leachate. Fine root demography has been shown to be quite plastic to variable conditions, including nutrient and/or chemical patches, water availability, soil depth, early root diameter, root age, season, and year [14, 20]. Furthermore, root mortality may coincide with patch depletion, and root quantity may be reduced as resources are exhausted or altered [20]. Therefore, just as plant roots increase growth given dynamic root responses to utilize areas of increased nutrients and water, plants also decrease growth rates and abscise tissue in response to

environmental stressors [18]. Currently, plastic responses were observed, enabling tissue avoidance to reduce exposure to the potentially toxic environment with leachate irrigation. Morphological plasticity governed fine root growth and reversed stress-driven necrotic responses, which were followed by tissue recovery at the same location as the necrosis. For example, environmentally induced callus cell proliferation extruding from the cutting lenticels was observed six times more often in leachate irrigated trees versus water; clonal trends were observed for genotypes of (*P. trichocarpa*×*P. deltoides*)×*P. deltoides* “TD×D” and *P. deltoides*×*P. suaveolens* subsp. *maximowiczii* “DM” parentage. In contrast, water-irrigated trees exhibited fine root clusters initiated from the same nodal origin five times more frequently than trees in the leachate treatment.

### Small Roots (2–5 mm)

Unlike fine roots, irrigation treatments (leachate chemistry, in particular) did not affect the production of small roots, which paralleled the overall response of young trees to high-salinity leachate [26, 32]. Puri et al. [21] tested *P. deltoides* at the end of a 9-year rotation that involved a traditional irrigation regime and three spacing treatments and found that there were no effects on small root biomass. In addition, small root biomass did not differ for percentage of total biomass, ranging from 4.4% to 7.2% [21].

Clones, however, differed for number of small lateral roots ( $P=0.0487$ ), which ranged from  $0.5\pm 0.5$  (DN5) to  $2.7\pm 0.5$  (NC14018) roots, with a mean of  $1.7\pm 0.2$  roots (Fig. 1). Genomic groups were affected differently, as the

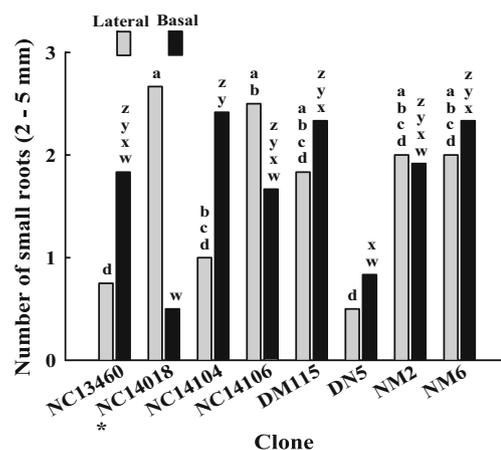


**Fig. 1** Number of small (2–5 mm) and coarse (>5 mm) lateral roots across water (control) and leachate irrigation treatments of eight *Populus* clones 14 months after planting. Each bar represents the mean of 12 trees with standard error=0.7 roots. Proportions of small and coarse lateral roots (standard error=0.6 roots) with different lowercase letters were different at  $P<0.05$ , whereas uppercase letters indicate clonal differences for total number of lateral roots

number of small lateral roots was significantly greater for NC14018 than NC13460 within the TD×D genomic group, while clones within other genomic groups did not differ for this trait. The TD×D backcross genomic group has frequently segregated for rooting and aboveground responses [27, 31]. For example, Friend et al. [7] conducted whole tree excavations on 2-year-old TD×D clones and found some roots to extend greater than 4 m horizontally and up to 1.5 m vertically from the cutting, as well as morphological differences in unbranched and highly branched lateral rooting. Small roots comprised 8% of total root biomass [7]. Overall, our results supported others that, although rooting is under strong genetic control, the genus *Populus* contains substantial genetic variability that results in genotypes differing in their plastic rooting responses across variable environments [3, 19, 31]. Comparisons across genomic groups highlighted clone NC14018, which had 2.7 and 5.4 times as many small lateral roots as NC14104 and DN5, respectively, supporting the assertion that selections can be conducted both within and among genomic groups. Furthermore, the clone×root type interaction was significant for the number of small roots ( $P=0.0068$ ), with only NC14018 exhibiting a difference in number of small basal and lateral roots (Fig. 2). Clone NC14018 had 5.3 times as many small lateral roots as small basal roots.

### Coarse Roots (>5 mm)

Coarse roots have comprised 26% of total root biomass for 2-year old TD×D genotypes [7]. In the current study, there were differences among irrigation treatments for a number



**Fig. 2** Number of small (2–5 mm) lateral and basal roots across water (control) and leachate irrigation treatments of eight *Populus* clones 14 months after planting. Each bar represents the mean of 12 trees with standard error=0.6 roots. Bars labeled with different letters were different at  $P<0.05$ . Root types were different for clone NC14018 (\*)

of coarse lateral roots ( $P=0.0225$ ), with trees receiving water having 30% more roots than those with leachate (Fig. 3). Additional cultural practices have affected coarse root production of *Populus*. Puri et al. [21] found that, regardless of spacing, the greatest coarse root biomass was located in the top 30 cm of soil, while root systems were more symmetrical at wider spacings. Although stand-level total coarse root biomass decreased with increased tree spacing, the total coarse root biomass and number of primary lateral roots increased on an individual-tree basis [21]. Furthermore, Coleman [1] found that fertilization and irrigation exerted less influence on coarse root biomass than spatial and temporal factors for four woody species, including *P. deltoides*. Clones in the current study differed for number of coarse lateral roots ( $P<0.0001$ ), which ranged from  $1.7\pm 0.6$  (NC13460) to  $5.7\pm 0.6$  (NM6) roots,

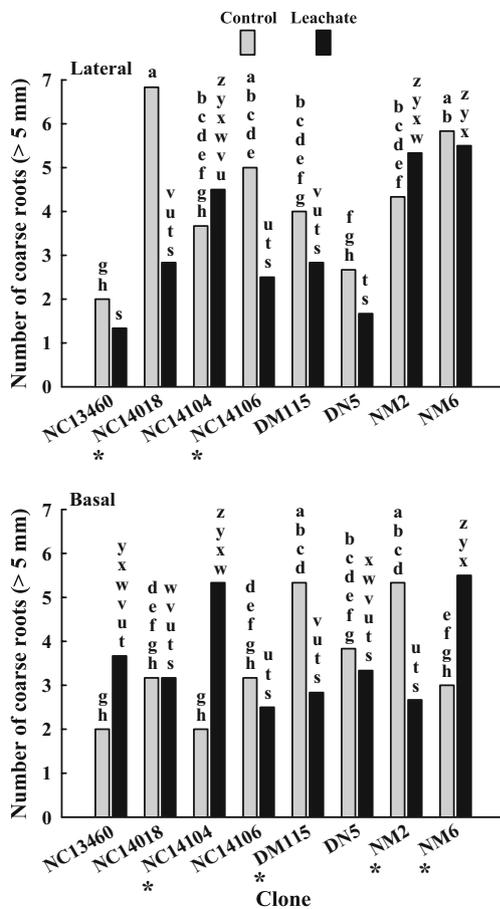
with a mean of  $3.8\pm 0.3$  roots (Fig. 1). The number of coarse lateral roots was significantly greater for NC14018 than NC13460 within the TD×D genomic group, while clones within other genomic groups did not differ for this trait. Clone NC14018 had 2.2 times as many coarse lateral roots as DN5, while NM6 significantly outperformed NC13460 (29% of NM6), NC14106 (66%), DM115 (60%), and DN5 (38%).

Similarly, clones differed for total number of lateral roots ( $P<0.0001$ ), which ranged from  $2.4\pm 0.7$  (NC13460) to  $7.7\pm 0.7$  (NM6) roots, with a mean of  $5.4\pm 0.3$  roots (Fig. 1). The total number of lateral roots was significantly greater for NC14018 than NC13460 within the TD×D genomic group, while clones within other genomic groups did not differ for this trait. Clone NC14018 had 1.5, 1.4, and 2.9 times as many total lateral roots as NC14104, DM115, and DN5, respectively. Similarly, NM6 significantly outperformed NC13460 (32% of NM6), NC14104 (66%), DM115 (68%), and DN5 (34%).

The treatment×clone×root type interaction was significant for number of coarse roots ( $P=0.0189$ ). Clones NC14018 and NC14106 exhibited a difference in number of coarse lateral roots between irrigation treatments (Fig. 3). The number of coarse lateral roots was 2.4 times greater for trees of clone NC14018 and two times greater for NC14106 irrigated with water versus leachate. Clones within the TD×D and DM genomic groups exhibited variable responses to irrigation treatments for number of coarse lateral roots, while rooting within other genomic groups was stable. Clones NC14104, DM115, NM2, and NM6 exhibited a difference in the number of coarse basal roots between irrigation treatments (Fig. 3). The number of coarse basal roots was 1.9 and two times greater for trees of clones DM115 and NM2, respectively, irrigated with water versus leachate. In contrast, the number of coarse basal roots was 2.7 and 1.8 times greater for trees of clones NC14104 and NM6, respectively, irrigated with leachate versus water. Clones within the DM and *P. nigra*×*P. suaveolens* subsp. *maximowiczii* “NM” genomic groups exhibited variable responses to irrigation treatments for number of coarse basal roots, while trends for other genomic groups were stable.

The clone main effect for coarse root diameter was significant ( $P=0.0459$ ), with diameter of DN5 greater than all other clones, which were similar to one another. Basal roots exhibited 8% greater coarse root diameter than lateral roots ( $P=0.0434$ ).

Deep, vertically oriented sinker roots present in the small and coarse root diameter classes developed on six of the eight clones tested, which may have contributed to increased anchorage and uptake potential. Likewise, Puri et al. [21] reported that sinker roots of *P. deltoides* penetrated the soil vertically and were more prevalent on trees planted 6×6 m apart compared to denser spacings. Additionally, the number and diameter of sinker roots



**Fig. 3** Number of coarse (>5 mm) lateral and basal roots of eight *Populus* clones 14 months after planting following once weekly landfill leachate irrigation during the 2005 (3.8 L per tree per week) and 2006 (22.7 L per tree per week) growing seasons. The control treatment was water applied at a volume equal to that of the leachate. Each bar represents the mean of six trees with standard error=0.8 roots. Bars labeled with different letters were different at  $P<0.05$ . Irrigation treatments were different within clones labeled with an asterisk (\*)

decreased with distance from the main root [21], which was similar to morphology of sinker roots observed in the current study. Friend et al. [7] observed that most trees of TD×D clones had one or two vertically oriented roots; this observation contrasted a greater number of sinker roots from 1-year-old trees of the same genotype. *Populus deltoides* has been shown to be highly responsive to non-uniform nutrient microsites, such as those related to irrigation drip tubes that may promote uneven root distribution and increased susceptibility to windthrow. Therefore, numerous, small-diameter, coarse roots may be used as selection criteria to bolster genotypes against windfall. *Populus* trees are also widely known as being deep-rooted, and this potential is maximized to strengthen anchorage for reduced windthrow [10] and to assist uptake during periods of drought stress and groundwater contaminant removal [6, 28]. However, only 9% of the young trees tested exhibited sinker roots. Therefore, in applications such as those listed above, it should be noted that only a small portion of the root system may be deep-rooted given that most of the belowground biomass is located near the soil surface [21].

#### Allometric Relationships

*Populus* species and hybrids have exhibited broad variation in allometric relationships among below- and aboveground traits for traditional applications [11, 12, 25] and phyto-technologies [27, 32]. Similarly, simple correlations among rooting traits for trees irrigated with water and leachate were highly variable in the current study (Table 3).

Correlations for water-irrigated trees ranged from  $-0.27$  (small basal roots with basal root dry mass) to  $0.92$  (stump dry mass with total root dry mass), while those for leachate-irrigated trees ranged from  $-0.53$  (small lateral roots with coarse basal roots) to  $0.96$  (stump dry mass with total root dry mass). Correlations among mass of lateral and basal roots were significant for both irrigation treatments; these results corroborated those reported previously for 1-year-old *P. deltoides* genotypes grown with a traditional water irrigation regime [12]. In contrast, Heilman et al. [12] reported non-significant correlations for lateral and basal root mass of *P. trichocarpa* and TD clones, which was similar to a lack of significance for all correlations among lateral and basal root numbers in the current study, with one exception: The number of small lateral and coarse basal roots was significantly negatively correlated (Table 3). Overall, correlations were greater for coarse roots than small roots. Small basal roots were weakly negatively correlated with dry mass traits, and coarse basal roots were significantly positively correlated with dry mass traits, regardless of irrigation treatment. In contrast, the strongest correlations for trees irrigated with water were among both number and dry mass traits with fine root scoring, while those for trees receiving leachate were mostly among number and dry mass traits. Similarly, Wullschleger et al. [25] reported significant positive correlations among mass of fine roots and coarse roots (measuring  $>2$  mm in diameter) for 1-year-old trees belonging to two advanced-generation pedigrees.

The distribution of small and coarse roots for both root types did not exhibit genomic group trends but was highly

**Table 3** Simple correlations ( $n=48$ ) among 12 rooting traits across eight *Populus* clones irrigated with water (above diagonal) and leachate (below diagonal)

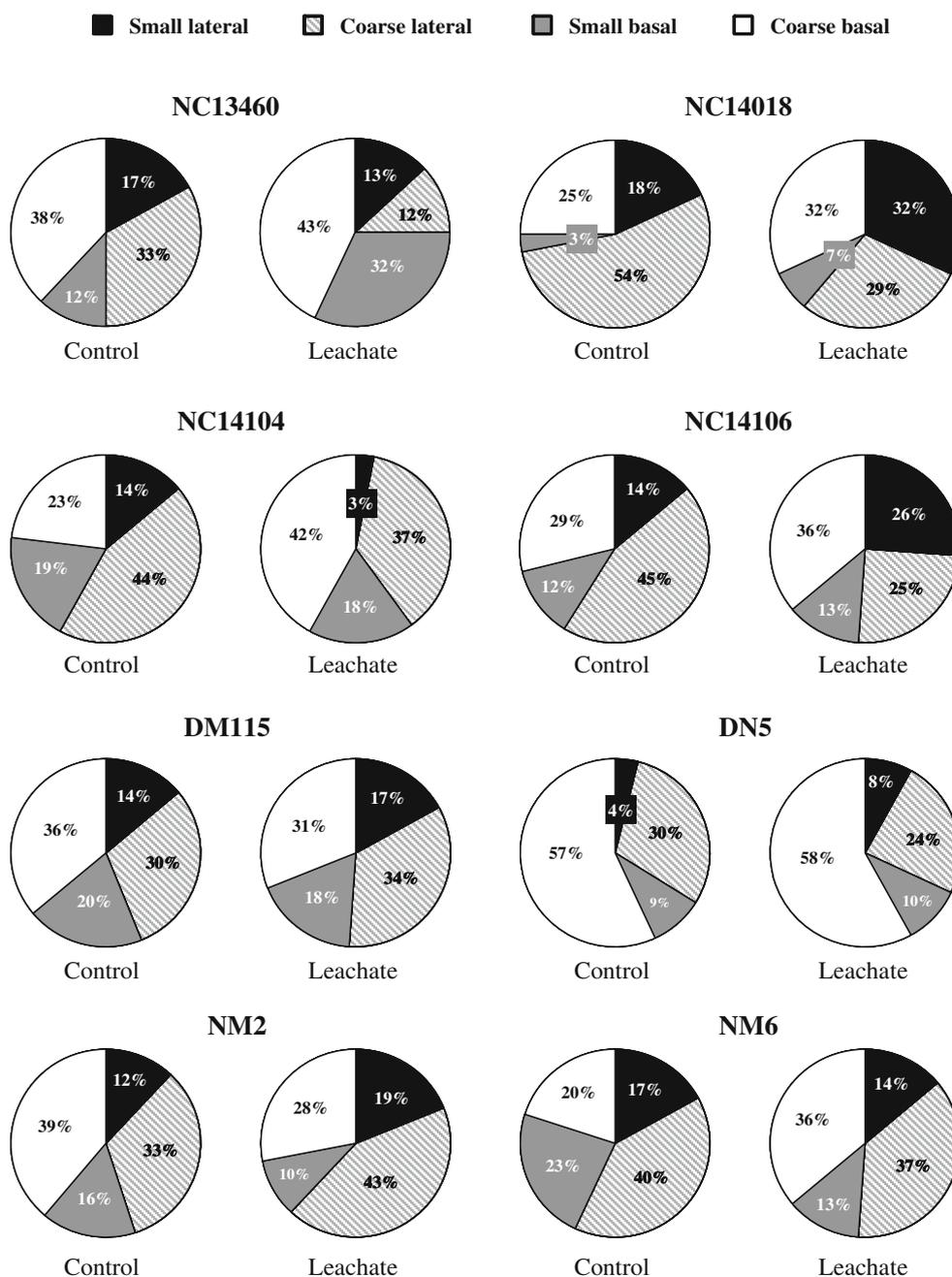
	Number of roots							Root dry mass				
	(1) Small lateral	(2) Coarse lateral	(3) Total lateral	(4) Small basal	(5) Coarse basal	(6) Total basal	(7) Total roots	(8) Fine root scoring	(9) Stump	(10) Lateral	(11) Basal	(12) Total
(1)		<i>-0.06</i>	<i>0.46</i>	0.21	0.05	0.16	<i>0.40</i>	<i>-0.04</i>	0.02	<i>-0.09</i>	<i>-0.17</i>	<i>-0.09</i>
(2)	0.01		<i>0.86</i>	<i>-0.01</i>	0.20	0.15	<i>0.65</i>	<i>0.39</i>	<i>0.52</i>	<i>0.80</i>	0.18	<i>0.59</i>
(3)	<i>0.73</i>	<i>0.69</i>		0.09	0.20	0.22	<i>0.78</i>	<i>0.32</i>	<i>0.47</i>	<i>0.66</i>	0.07	<i>0.47</i>
(4)	0.18	0.11	0.21		<i>-0.03</i>	<i>0.60</i>	<i>0.44</i>	<i>0.29</i>	<i>-0.06</i>	<i>-0.10</i>	<i>-0.27</i>	<i>-0.16</i>
(5)	<i>-0.53</i>	0.15	<i>-0.28</i>	0.06		<i>0.79</i>	<i>0.63</i>	0.21	<i>0.57</i>	0.16	<i>0.53</i>	<i>0.46</i>
(6)	<i>-0.26</i>	0.17	<i>-0.07</i>	<i>0.69</i>	<i>0.76</i>		<i>0.78</i>	<i>0.35</i>	<i>0.42</i>	0.07	0.26	0.27
(7)	<i>0.38</i>	<i>0.65</i>	<i>0.72</i>	<i>0.64</i>	<i>0.31</i>	<i>0.64</i>		<i>0.43</i>	<i>0.57</i>	<i>0.47</i>	0.21	<i>0.48</i>
(8)	<i>0.31</i>	0.16	<i>0.34</i>	0.24	<i>-0.18</i>	0.03	0.28		<i>0.41</i>	<i>0.50</i>	<i>0.32</i>	<i>0.47</i>
(9)	<i>-0.29</i>	0.24	<i>-0.05</i>	<i>-0.20</i>	<i>0.62</i>	<i>0.32</i>	0.18	<i>-0.27</i>		<i>0.65</i>	<i>0.79</i>	<i>0.92</i>
(10)	<i>-0.09</i>	<i>0.74</i>	<i>0.44</i>	0.03	<i>0.36</i>	0.28	<i>0.54</i>	<i>-0.01</i>	<i>0.60</i>		<i>0.49</i>	<i>0.84</i>
(11)	<i>-0.40</i>	0.04	<i>-0.27</i>	<i>-0.20</i>	<i>0.69</i>	<i>0.36</i>	0.05	<i>-0.20</i>	<i>0.88</i>	<i>0.42</i>		<i>0.86</i>
(12)	<i>-0.31</i>	<i>0.35</i>	0.01	<i>-0.15</i>	<i>0.65</i>	<i>0.37</i>	0.27	<i>-0.20</i>	<i>0.96</i>	<i>0.74</i>	<i>0.90</i>	

Correlations in italics were significant at  $P<0.05$ . See “Materials and Methods” for descriptions of rooting traits

clone specific (Fig. 4). With the exception of clone DM115, the proportion of roots allocated to each combination of root type and size changed when trees were irrigated with leachate versus water. Overall, leachate affected lateral roots more than basal roots. Leachate caused shifts in allocation of root sizes and types ranging from -79% (NC14104, small laterals) to +167% (NC13460, small basals), with the least amount of change being +2% for coarse basal roots of clone DN5. The number of small lateral roots increased with leachate compared to water, while there were fewer coarse lateral roots. In contrast, the number of small basal roots decreased with leachate

compared to water, while coarse basal roots increased. The overall lack of trends across genomic groups contradicts results of Heilman et al. [12] who reported greater numbers of lateral than basal roots for *P. deltoides*, *P. trichocarpa*, and TD genotypes. They reported a twofold increase in number of lateral versus basal roots for *P. deltoides* [12], while threefold increases are reported here for specific clones, but there are no increases among genomic groups (Fig. 4). Such clonal variation in rooting and other traits has been reported often and is largely proportional to the broad genetic differences among parental species tested [24].

**Fig. 4** Mean proportion (%;  $n=6$ , based on number of roots) of small (2–5 mm) and coarse (>5 mm) roots of eight *Populus* clones 14 months after planting following once weekly landfill leachate irrigation during the 2005 (3.8 L per tree per week) and 2006 (22.7 L per tree per week) growing seasons. The control treatment was water applied at a volume equal to that of the leachate. Lateral roots developed from latent root primordia distributed throughout the length of the cuttings, while basal roots developed from callus at the base of the cuttings



## Practical Implications

Root growth in young *Populus* plantations is highly correlated with aboveground biomass production and is crucial for long-term stand performance [12, 19, 21]. There is an opportunity to use knowledge of lateral and basal adventitious roots to make improved genotypic selections and enhance plantation success for commercial applications such as feedstock production for biofuels, bioenergy, and bioproducts, as well as ecological applications such as phytotechnologies and riparian buffer systems [25, 30]. In the current study, we examined variable basal and lateral root numbers of eight *Populus* clones following two growing seasons with leachate and water irrigation. Generally, leachate irrigation did not negatively affect overall root growth, which corroborated our previous studies that reported minimal foliar disruption of aboveground productivity due to high-salinity leachate chemistry [27–29]. Overall, these combined results support continued testing and wastewater reuse irrigation projects, given the potential to select favorable clones for specific wastewater constituents and sites (specialists) or those that exhibit optimal growth across heterogeneous conditions (generalists) [31]. Information about the variation in morphology and development of lateral and basal adventitious rooting of *Populus* will help with such genotypic selection, as well as continued development of silvicultural systems that utilize rooting as a key trait for improved plantation performance [3].

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